RMUTL. Eng. J

Design and Analysis of an SME-Level Pulsed Electric Field Device for Extracting Bioactive Compounds from Black Rice

Supakiat Supasin¹, Panich Intra², Pornsawan Sombatnan², Sureewan Rajchasom², Padipan huangsorn¹, Thanachat Mahawan³ and Chatchawan Kantala^{2*}

Abstract

This research developed a small-scale Pulsed Electric Field (PEF) machine to extract bioactive compounds from black rice grown in Doi Saket and assessed its extraction efficiency. The primary voltage ranged from 0 to 220 V, with secondary high voltage AC and DC outputs spanning from 0.68 to 15.00 kV and 0.96 to 21.21 kV, respectively. The experiment used a ratio of 1 kg of black rice to 2 L of water, with electric field strengths of 4, 5, and 6 kV/cm at a frequency of 1 Hz, varying the number of pulses between 1,000, 3,000, and 5,000. Results showed that 6 kV/cm and 5,000 pulses yielded the highest anthocyanin content $(3.23\pm0.04 \text{ mg/L})$, which significantly differed from other conditions (p<0.05). The highest antioxidant levels were observed at 4 kV/cm for 1,000 pulses and 5 kV/cm for 1,000 pulses (77.86±0.67% and 76.91±0.71%, respectively), though these levels decreased in comparison to traditional extraction, showing statistical significance (p<0.05). However, a higher pulse count led to an increase in anthocyanin content. Furthermore, increased electric field intensity raised antioxidant yields, though this effect plateaued beyond a certain point. Optimal extraction conditions were achieved at 5 kV/cm and 3,000 pulses, yielding anthocyanin and antioxidant contents of 1.02±0.04 mg/L and 59.72±0.34%, respectively. The extraction process was most effective when temperatures remained below 50°C (without a cooling system) and pressure was kept at 1 atm. Additionally, the study developed a PEF prototype for bioactive compound extraction from black rice.

Keywords: Pulsed Electric Field, Extraction, Black Rice, Bioactive Compounds, Anthocyanin, Antioxidant

1. Introduction

Khao Gam Doi Saket is a variety of sticky rice known for its black color. The name "Doi Saket" comes from the area where it is grown, specifically in the village of San Pu Loei, San Pu Loei Sub-district, Doi Saket District, Chiang Mai Province. In addition to being grown in Chiang Mai, Khao Gam is also found in other provinces and regions, such as the central region, where it is commonly known as "black sticky rice." In the southern region, it is referred to as "Niaw Dam" or sometimes as "Khao Nil" due to the dark red or deep red color of the grain. There

are several varieties of Khao Gam in Thailand, and in Chiang Mai Province, specifically, the Doi Saket Campus of Rajamangala University of Technology Lanna is home to a group of farmers cultivating the Doi Saket variety of Khao Gam. This variety has been registered and certified by the Department of Agriculture under the Plant Varieties Protection Act B.E. 2518 (1975). The rice variety was improved in 1995 by the Institute of Science and Technology Research at Chiang Mai University [1]. Khao Gam is recognized for its medicinal and herbal properties. It was found that Khao Gam Doi

¹ Faculty of Engineering, Rajamangala University of Technology Lanna, 128 Huay Kaew Road, Chang Phueak, Mueang Chiang Mai, Chiang Mai, Thailand, 50300.

² Research Unit of Applied Electric Field in Engineering (RUEE), College of Integrated Science and Technology, Rajamangala University of Technology Lanna 98 Moo 8, Pa Pong, Doi Saket, Chiang Mai, Thailand, 50220.

³ Office of Facilities and Services Management, Payap University, 272 Moo 2, San Phanet, San Sai, Chiang Mai, Thailand, 50210.

^{*}Corresponding author: chatchawan_kantala@yahoo.com, Telephone number: +66-642974595 Received: 1 January 2025, Revised: 24 November 2025, Accepted: 27 November 2025

Saket contains Gamma Oryzanol, which is three times higher than that in white rice. Additionally, it contains dark blue to purplish-black pigments, Proanthocyanidin (Cyanidin glucoside), Anthocyanin, and is rich in vitamin E [2]. Khao Gam Doi Saket is promoted for cultivation in the Doi Saket District of Chiang Mai Province. A group of farmers in the village of San Pu Loei, San Pu Loei Sub-district, Doi Saket District, have come together to grow this rice on an area of 20 rai, yielding 750 kg per rai. The cultivated area is expected to expand, with more farmers showing interest in joining the group each year. Currently, the group sells milled rice to private companies. However, the group sees great potential in processing Khao Gam into various products, which would add value and create jobs for people in the village. As a result, a group of female farmers has been established to process products from Khao Gam. At present, the group has no products because they have not received support from any organizations, nor do they possess the necessary knowledge.

Khao Gam Doi Saket is rich in essential nutrients, including carbohydrates, fats, proteins, vitamins A, B1, B2, E, calcium, and iron. It also contains high levels of flavonoids antioxidant compounds that surpass those in white rice [3]. Flavonoids, a type of polyphenol, are composed of 15 carbon atoms arranged in an aromatic ring structure with hydroxyl groups. More than 4,000 types of flavonoids exist in nature, predominantly in the glycoside form. Key flavonoids include flavanol, flavanone, flavone, isoflavone, catechins, and anthocyanins [4]. Further studies revealed that Gamma Oryzanol, when working in conjunction with Anthocyanin, has antioxidant properties that help slow down aging, prevent cancer, obesity, hypertension, heart disease, and memory loss, boost immunity, and lower cholesterol levels [5].

Anthocyanins and gamma-oryzanol, found in Khao Gam, are pigments ranging from red to purple or blue. Anthocyanins are watersoluble, pH-sensitive compounds that belong to the flavonoid group [6]. Their color changes with pH: red in acidic conditions, purple at

neutral pH, and blue in alkaline conditions. These pigments are stored in the vacuole sap and play a protective role against environmental stressors. However, they are prone to degradation due to high temperatures, oxygen, and sugar concentration [7]. Anthocyanins are widely applied for their antioxidant properties in various industries, including food, cosmetics, and health products. Their chemical structure includes aglycone, sugar, and acyl groups, with 15 carbon atoms per molecule. Common anthocyanidins include cyanidin, peonidin, and others, contributing to the distinctive characteristics of Khao Gam [8, 9].

The major chemical composition of Khao Gam Doi Saket is anthocyanin, which is sensitive to heat extraction. Conventional extraction methods, such as boiling, Soxhlet extraction, and maceration, have been employed for centuries. However, these traditional techniques are often timeconsuming, energy-intensive, and involve high temperatures, which can lead to the degradation of heat-sensitive nutrients [10]. To overcome these limitations, emerging extraction technologies that operate under mild or cold processing conditions are being explored to enhance anthocyanin yield and preserve nutritional quality. The pulse electric field extraction (PEF) is well known for the extraction of pure dry matter, carotenoids, vitamins, sucrose, proteins, inulin, and other substances [11]. This technique offers a promising alternative to traditional methods, particularly for thermolabile compounds such as anthocyanins. PEF is a non-thermal technology that uses high-voltage pulses (1-20 us) at intensities of 20-50 kV/cm to induce electroporation, making it effective applications such as extraction, microbial inhibition, and pickling. The process operates without heat or chemicals, preserving the taste, aroma, color, and nutritional value of food products. A typical PEF system comprises three components: a DC power supply to convert AC into high-voltage DC, a pulse modulator to generate pulsed electric energy, and a treatment chamber where samples are exposed to the



electric field. The efficiency of PEF depends on factors like pulse shape, conductivity, electric field strength, and frequency [12, 13]. Eshtiaghi and Knorr [14] reported enhanced sugar extraction from beetroot using PEF (1.2-2.5 kV/cm), while Rastogi et al. [15] demonstrated improved mass transfer and cell disruption in carrots. Bobinaite et al. [16] observed a 60% increase in anthocyanin content and a 31% rise in antioxidant activity in PEF-treated blueberries. Similarly, Zhi-Hong et al. [17] found enhanced pigment and antioxidant retention in spinach. Francisco et al. [18] reported that PEF increased total polyphenol content and antioxidant capacity in borage by up to 13.7-fold. Moreover, PEF has also been successfully applied in combination with freeze-thaw techniques to enhance pigment extraction efficiency from cyanobacteria [24]. peaches, In **PEF** significantly improved the extraction of phenolics and flavonoids using only water as a solvent [19]. Overall, PEF offers advantages over conventional thermal methods, including reduced processing time, lower energy input, and better preservation of thermolabile compounds, making it a sustainable and efficient approach for extracting sensitive phytochemicals such as anthocyanins.

Therefore, based on the nutritional properties of Khao Gam and its ability to enhance immunity against various diseases, as well as the fact that the pigments in Khao Gam can be extracted using advanced technology without destroying their beneficial properties, it can be developed into health products, cosmetic products, or other related items. Although PEF technology has been widely studied and applied internationally for the extraction of bioactive compounds in various agricultural products, most existing research and commercial applications are concentrated in large-scale or industrial settings. In Thailand, however, the adoption and development of PEF technology at the small and medium enterprise (SME) level remain limited. There is a noticeable lack of localized studies focusing on the design, optimization,

and practical implementation of PEF systems that are affordable, scalable, and tailored to the needs of Thai SMEs, particularly for the extraction of valuable compounds from indigenous crops such as Khao Gam. The project has thus been designed to analyze and develop a pulse electric field (PEF) machine for SMEs to extract bioactive compounds from Khao Gam. The project aims to test the extraction efficiency of the PEF machine, study the optimal electric field intensity, and determine the appropriate number of pulses for extracting bioactive compounds from Khao Gam, in comparison with the traditional extraction method.

2. Research methodology

2.1 Design and construction of an extraction machine.

The power module consisted of a high-voltage transformer, rectifier, and pulse-forming network with solid-state switching, similar to designs reported by Kantala et al. (2022) [12]. The PEF generator produced monopolar square-wave pulses with a pulse width of 2.5 µs, a fast rise time (<500 ns), and a frequency of 1 Hz, ensuring effective electroporation while minimizing thermal effects.

The design and construction of the extraction machine began with a study of theoretical concepts, a review of relevant Literature, and the design of subcomponents for the PEF machine and treatment chamber. The treatment chamber, with a 500 mL capacity, features two flat stainless-steel electrodes (316L) separated by food-grade Teflon insulation. The process included creating a prototype, drafting detailed plans, and sourcing materials and equipment for assembly. Each component was tested individually before assembling the complete PEF machine. The system was then tested for its performance in extracting bioactive compounds from black rice, and the results were analyzed. Table1 outlines the system specifications.

Table 1. Properties of the bioactive compound extraction system to be developed for black rice

extraction system to be developed for black fice		
Design	Specifications	
Conditions		
Principle	Electroporation	
Size	SME	
Type of food	Black rice	
Electric field	> 2 kV/cm	
strength		
Dimensions of the	Gap: 4.0 cm and 500 ml	
black rice extraction		
chamber		
Voltage at electrode	≤ 20 kV	
poles	\leq 2 kA at a pulse width	
Maximum output	of approximately 2.5 µs	
current		
Maximum output	$\leq 2 \text{ kA}$	
current		
Pulse width	~ 2.5 µs	
Pulse frequency	1-5 Hz	
Voltage	Positive electrode	
Working fluid	1 bar	
pressure		
Production capacity	At least 5,000 ml/hr of	
	bioactive composite extract	
Efficiency	Better extraction of bioactive	
·	compounds from black rice	
	compared to traditional	
	extraction methods	
Maintenance	Easy to disassemble, clean,	
	and install	

2.2 Testing the efficiency of bioactive compound extraction from black rice using the PEF machine.

The efficiency of bioactive compound extraction from black rice using the PEF machine was evaluated by selecting and washing black rice, followed by extracting with the PEF machine. A rice-to-water ratio of 1:2 was maintained, with adjustments to the electric field intensity at 4, 5, and 6 kV/cm at a frequency of 1 Hz. The number of pulses was varied between 0 (Control), 1,000, 3,000, and 5,000 pulses, respectively. These results were compared to those from the conventional extraction method for bioactive compounds in black rice. The process for testing bioactive compounds from black rice is illustrated in Figure 1.

2.3 Black Rice

The black rice used for extraction was sourced from the Doi Saket community enterprise and is characterized by its dark purple or black stems, purple husks, and grains measuring approximately 3.3 mm wide, 9.7 mm long, and 1.91 mm thick Figure 2. Bioactive compounds were extracted using a PEF machine, and the results were analyzed both before and after extraction. Physical properties, including L^* , a^* , and b^* values, were measured in triplicate, and data were processed in Microsoft Excel with statistical analysis performed using the MINITAB ANOVA-t test. Chemical properties, such as total anthocyanin content and antioxidant activity, were also evaluated under the same statistical protocol.

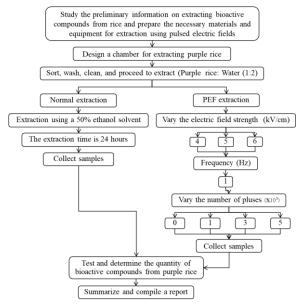


Figure 1 Experimental procedure

The initial experimental ranges (4-6 kV/cm; 1,000-5,000 pulses; 1 Hz) were selected based on prior PEF Literature, which typically reports effective electroporation at 1-20 kV/cm and 500-10,000 pulses. These parameters were chosen to ensure safe operation suitable for SME-scale equipment while covering an effective electroporation window for bioactive compound extraction.

2.4 Study of Optimal Conditions for Pulsed Electric Field Extraction Process

The extraction of bioactive compounds from black rice is performed using two methods: solvent extraction and pulsed electric field extraction. For the solvent extraction, the ratio used is 1 kg of purple rice to 2 L of water. Water and 50% ethanol are used as solvents, and the extraction time is 24 hours. Response surface



methodology (RSM) was used for optimization of PEF conditions. The 2-factor factorial design was used in designing the PEF treatment. For the pulsed electric field extraction, experiments are conducted with electric field strengths of 4, 5, and 6 kV/cm at a frequency of 1 Hz, and the number of pulses is varied at 1,000, 3,000, and 5,000 pulses, respectively. The model regression was calculated for the optimum point by the MINITAB program.



Figure 2 Doi Saket Black Rice Variety

2.5 Analysis of the Chemical Properties, Bioactive Compounds, and Key Components of the Concentrated Black Rice Beverage Extract

After measuring the physical properties, including color, the concentrated extract sample from Doi Saket black rice was analyzed for its chemical properties and bioactive compounds. These include total flavonoid content, anthocyanin content, antioxidant activity, and the levels of cyanidin and peonidin. The analysis details are as follows:

The analysis of total flavonoid content was performed using the aluminum chloride colorimetric method with quercetin as the standard. The total flavonoid content was determined by comparing it to a standard concentration graph (ranging from 10-100 mg/ml). A 1 mL sample was taken, and 1 mL of 10% aluminum chloride was added, followed by shaking the mixture. Then, 1 M potassium acetate was added, and the mixture was shaken again. The sample was kept in the dark for 45 minutes, after which the absorbance was measured using a spectrophotometer at a wavelength of 415 nm. The flavonoid content in the extract was calculated by comparing it to the quercetin standard graph (Y $= ...x + ..., R^2 = 0.999$) and expressed as milligrams of quercetin equivalent per gram of extract (mg of quercetin equivalent/g extract) [20].

The analysis of anthocyanin content was performed using the pH differential method. A 1 mL sample was taken and diluted with distilled water to a final volume of 10 ml. The mixture was shaken well and centrifuged at 3,000 rpm for 15 minutes. Only the clear supernatant was collected, and 3 mL of it was diluted with pH 1 and pH 4.5 buffer solutions to a final volume of 30 ml. The solution was left to stand for 30 minutes, after which the absorbance was measured using a spectrophotometer at wavelengths of 510 nm and 700 nm [21]. Anthocyanin content was calculated based on the absorbance of the solution using the appropriate formula:

The total anthocyanin content. = $\frac{\text{Adiff} \times \text{MW} \times \text{df} \times 1000}{\epsilon}$

where, Adiff = (A510-A700) pH1- (A510-A700) pH4.5, Mw = 449.2 g mol, $\epsilon = 26900$ M cm, dilution factor (df) = 1.

The analysis of antioxidant activity was performed using the DPPH radical scavenging assay. A 0.12 mM standard solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) was prepared. For the test sample, 200 µl of the sample was mixed with 1.8 mL of methanol and 2 mL of DPPH solution. For the control, 2 mL of methanol was mixed with 2 mL of DPPH solution, and for the blank, 4 mL of methanol was used [22]. The antioxidant activity was calculated using the percentage inhibition formula:

The percentage of inhibition = $\frac{(A517_{control} - A517_{sample}) \times 100}{A517_{control}}$

Where, A517_{control} = absorbance of control sample at 517 nm, A517_{sample} = absorbance of test sample at 517 nm.

The analysis of cyanidin and peonidin content was performed using High Performance Liquid Chromatography (HPLC) with a C1 8 column. Mobile phase A consisted of water and formic acid in a 90:10 v/v ratio, and Mobile phase B consisted of water, acetonitrile, methanol, and formic acid in ratios of 40:22, 5:22, and 5:10 v/v. The ratio of Mobile phase A to Mobile phase B was 50:50 v/v, with a flow rate of 1

ml/min. Detection was performed at a wavelength of 5 3 5 nm, with an injection volume of 20 μ l. The analysis was carried out at a temperature of 4 °C, and the column temperature was maintained at 30°C [23].

2.6 Statistical Analysis of Experimental Results

Statistical analysis of the data was performed by analyzing the physical and chemical results from each experiment, with all analyses conducted in triplicate. Data were expressed as mean \pm standard deviation (mean \pm SD) using MINITAB Statistical Software version 20

3. Results and Discussion

3.1 Results of the Design, Construction, and Testing of the PEF Machine

3.1.1 Design Results

The design of a Pulse Electric Field Machine for SMEs to develop a Process for Extracting Bioactive Compounds from Black Rice, using SolidWorks software as shown in Figure 3.

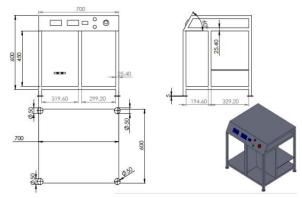


Figure 3 The pulsed electric field (PEF) device schematic drawing diagram.

3.1.2 Construction Results of the PEF Machine

A prototype PEF machine was constructed with a maximum voltage of 20~kV, a frequency range of 1-5~Hz, a pulse width of approximately $2.5~\mu s$, and an electric field intensity greater than 2~kV/cm, as shown in Figure 4, which was designed for extracting bioactive compounds from black rice.



Figure 4 Internal components of the PEF device.

3.1.3 Performance Testing Results of the PEF Machine

The electrical performance of the pulse power supply was evaluated by measuring the primary and secondary voltages of the high-voltage transformer across the operating range. Table2 summarizes the energy requirements at different electric field strengths (4, 5, and 6 kV/cm) and pulse numbers (1,000, 3,000, and 5,000). Table3 further presents the relationship between the primary voltage, high-voltage AC output, and rectified DC voltage of the transformer module. The primary voltage was varied from 0 to 220 V, demonstrating that energy consumption increased proportionally with both electric field intensity and pulse count.

Table 2 Results of the relationship between electric field intensities, number of pulsed, and energy.

field intensities, number of pulsed, and energy.			
Electric Field Intensities (kV/cm)	Number of Pulsed (Pulsed)	Energy (kJ/L)	
4	1,000	160	
	3,000	480	
	5,000	800	
5	1,000	250	
	3,000	750	
	5,000	1,250	
6	1,000	360	
	3,000	1,080	
	5,000	1,800	



Table 3 Results of the relationship between primary voltage, secondary high AC voltage, and high DC output voltage.

Primary	High Secondary	High Output
	Alternating	Direct Voltage
Voltage (V)	Voltage (kV)	(kV)
0	0.06	0.09
10	0.68	0.96
20	1.36	1.93
30	2.05	2.89
40	2.73	3.86
50	3.41	4.82
60	4.10	5.79
70	4.77	6.75
80	5.46	7.71
90	6.36	8.68
100	6.82	9.64
110	7.50	10.61
120	8.18	11.57
130	8.86	12.53
140	9.55	13.50
150	10.23	14.46
160	10.91	15.43
170	11.60	16.39
180	12.27	17.35
190	12.96	18.32
200	13.64	19.28
210	14.32	20.25
220	15.00	21.21

The purpose of Table 3 is to verify the transformer step-up ratio and DC output stability of the custom pulse power module, serving as an indirect indicator of delivery efficiency. Due to proprietary technical constraints, only input-output characterization is reported; however, the results are consistent with previously reported PEF power system performance [12], supporting the reliability of the design for SME-scale operation. 3.2 Results of the Extraction Conditions Study for

3.2.1 Physical Characteristics of Doi Saket Black Rice Extract

Doi Saket Black Rice

The black rice extracts obtained using plain water extraction and PEF extraction methods are shown in Figure 5. The physical characteristics of the black rice extract are liquid with a deep red to dark purple color. This is due to the presence of anthocyanin pigments in the black rice grains, which are highly soluble in water.

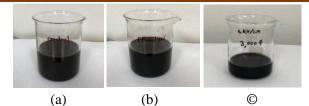


Figure 5 Physical characteristics of black rice extract: (a) Control; (b) 5 0 % Ethanol; (c) Extracted with PEF.

The distinct coloration observed in the PEF-treated extract reflects the efficient release of anthocyanin pigments from the rice matrix. This outcome aligns with the applied PEF waveform characteristics (monopolar square-wave, 2.5 µs pulse width, <500 ns rise-time), which promote electroporation-driven cell disruption while minimizing thermal effects. Consequently, thermolabile bioactive compounds are better preserved during extraction.

3.2.2 Percentage Yield and pH Analysis Results of Black Rice Extract

In the study of optimal conditions for PEF extraction of black rice, pulse numbers of 1,000, 3,000, and 5,000 were tested and compared with 50% ethanol extraction over 24 h [25]. Ethanol extraction yielded a total anthocyanin content of 1.59 ± 0.16 mg/L. The highest anthocyanin content, 3.23 ± 0.04 mg/L, was achieved with PEF at 6 kV/cm and 5,000 pulses, significantly higher than all other methods with 95% confidence (p<0.05). Antioxidant activity was highest at 4 kV/cm and 1,000 pulses (77.86 \pm 0.67%) and 5 kV/cm and 1,000 pulses (76.91 \pm 0.71%), both significantly different from other conditions at 95% confidence (p<0.05).

The optimal conditions for extracting valuable compounds from Doi Saket black rice were achieved using PEF at 5 kV/cm with 5,000 pulses, yielding a total anthocyanin content of 2.58 ± 0.04 mg/L and antioxidant activity of $54.77 \pm 3.48\%$, as shown in Figure 6. Compared to plain water extraction (Control), PEF extraction produced higher levels of bioactive compounds in less time. Higher voltages and pulse numbers generally increased anthocyanin content, with more pulses resulting in higher yields. However, increased extraction energy reduced antioxidant activity, likely due to heat generation during extraction,

which may diminish antioxidant effectiveness [26]. Several studies support the observation that high electric field intensity and excessive pulse numbers in PEF treatment can lead to a decline in antioxidant activity. Mahnič-Kalamiza et al. [27] noted that excessive energy input during electroporation could cause the degradation of bioactive compounds due to local heating and membrane damage. Similarly, Zhang et al. [28] reported that when extracting pigments from spinach, field intensities beyond the optimal range reduced antioxidant yield, likely due to structural instability. In blueberry processing, Pataro et al. [29] also found that increasing the field strength beyond 1 kV/cm did not improve antioxidant content and could potentially cause compound degradation. These findings align with the current study's results, emphasizing the importance of optimizing PEF conditions to maximize bioactive compound retention without compromising antioxidant integrity.

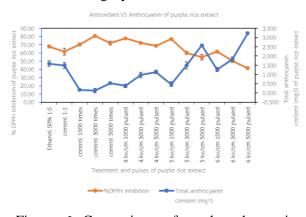
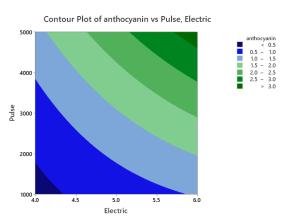


Figure 6 Comparison of total anthocyanin content (mg/L) and antioxidant activity from extractions at electric field intensities of 4, 5, and 6 kV/cm with 1,000, 3,000, and 5,000 pulses.

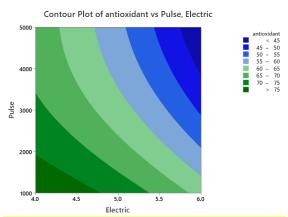
3.2.3 Analysis of Optimal Extraction Factors for Black Rice Using Response Surface Methodology (RSM)

Based on the experimental results, the optimal conditions for extracting black rice were determined to be a voltage of 5 kV/cm with 5,000 pulses. As shown in Figure 6, higher electric field intensity and pulse numbers increased anthocyanin content, whereas antioxidant activity tended to decrease. This indicates that although stronger PEF treatment enhances pigment release,

excessive energy input may promote degradation of antioxidant compounds though local heating. These findings are consistent with previous studies reporting reduced antioxidant stability under high-intensity PEF [30, 31]. Thus, the results highlight the need for optimization to balance anthocyanin yield and antioxidant retention. The Response Surface Methodology from MINITAB software was used to further refine the extraction conditions. The analysis revealed that a voltage of 6 kV/cm and 5,000 pulses resulted in the highest total anthocyanin content of 3.25 mg/L, while extraction at 4 kV/cm with 1,000 pulses provided the highest antioxidant activity of 79.64%, as shown in Figure 7.



(a) RSM results for the total anthocyanin content of black rice extract.



(b) RSM results for the antioxidant activity of black rice extract.

Figure 7 Response Surface Methodology (RSM)

Subsequently, the Response Optimizer function was utilized to identify the optimal factors that provide consistent conditions for extracting both the total anthocyanin content and antioxidant



activity. The optimal extraction conditions determined were a voltage of 4.63 kV/cm and 5,000 pulses, yielding a total anthocyanin content of 1.99 mg/L and an antioxidant activity of 61.10%, as depicted in Figure 8. The conditions that were closest to achieving both high total anthocyanin content and antioxidant activity involved using a voltage of 5 kV/cm with 3,000 pulses, resulting in a total anthocyanin content of 1.49 ± 0.19 mg/L and an antioxidant activity of $60.13 \pm 1.95\%$, respectively.

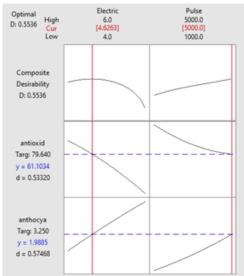


Figure 8 Optimal conditions for black rice extraction based on the optimal values of total anthocyanin content and antioxidant activity.

3.2.4 Cost-Benefit Consideration and SME Scalability

solvent Compared with conventional extraction, the PEF method substantially shortens the processing time (from ~24 h to only a few minutes) and eliminates solvent costs by utilizing water as the extraction medium. Although high voltage is required, the energy consumption per batch remains low due to the short pulse duration and low-frequency operation, with an estimated energy use of approximately 0.15-0.20 kWh per kg of black rice. Additionally, the PEF system is compact, low-maintenance, and suitable for SMEs. Importantly, the developed PEF machine achieved comparable or higher anthocyanin yields demonstrating within minutes, significant advantages in both processing efficiency and solvent reduction relative to traditional extraction.

Overall, these findings indicate that PEF technology offers a scalable, energy-efficient, and solvent-free extraction strategy suitable for SME-level production of functional foods and bioactive compounds.

4 Conclusions

The extraction machine designed for Doi Saket black rice effectively extracted anthocyanins under optimal conditions of 5 kV/cm with 3,000 pulses or 4.63 kV/cm with 5,000 pulses, as determined by the Response Optimizer. The PEF machine allows for primary voltage adjustments from 0 to 220 V, producing secondary high voltages of 0.68-15.00 kV and DC output voltages of 0.96-21.21 kV. During extraction, the temperature remained below 50°C without cooling, at room temperature, and 1 atm pressure. This SME-level PEF machine demonstrates high efficiency in bioactive compound extraction, particularly anthocyanins, from black rice. When compared with 24-hour conventional maceration, the optimized PEF conditions produced comparable or superior within while anthocyanin vields minutes, significantly lowering solvent usage and processing time. Collectively, this work the technical feasibility demonstrates industrial relevance of PEF as a rapid, energyefficient, and solvent-free extraction technology for SME-level production of bioactive-rich functional ingredients.

5. Acknowledgements

The results of this research and development project were funded by the Thailand Science Research and Innovation (TSRI) for the fiscal year 2021, Basic Research Fund (Contract number; 2564Basic011). We would like to thank Mr. Surapol Sudakaew, President of the Doi Saket Black Rice Community Enterprise, Materials and Medical Innovation Research Unit, Faculty of Engineering, RMUTL, and Research Unit of Applied Electric Field in Engineering (RUEE), College of Integrated Science and Technology Rajamangala University of Technology Lanna during the project.



6. References

- [1] Lanna Rice Research center. Khoa Gam Lanna. 2022. Available from: https://lannarice.cmu.ac.th/pages/view/24. [Accessed 12 January 2024].
- [2] Fongfon S, Pusadee T, Prom-u-thai C, Rerkasem E, Jamjod S. Diversity of Purple Rice (Oryza sativa L.) Landraces in Northern Thailand. Agronomy. 2021;11:2029.
- [3] Nuananong S, Nakamon K, Weeraphong C. Flavonoid content, total phenolic compounds and their anti-free radicals of Thai rice extracts. 2001. Available from: http://www.research.cmru.ac.th/research59/ris/view.php?no=545. [Accessed 12 January 2024].
- [4] Narikawa T, Shinomiya H, Fuji T. A betarutinosidase from Penicillium rugulosum IFO 7242 that is a peculiar flavonoid glycosidase. Biosci Biotechnol Biochem. 2000; 64:1317-19.
- [5] Patras A, Brunton NP, O'Donnell C, Tiwari BK. Effect of thermal processing on anthocyanin stability in foods; mechanisms and kinetics of degradation. Trends Food Sci Technol. 2010:3-11.
- [6] Lazze MC, Savio M, Pizzala R, Cazzalini O, Perucca P, Scovassi AI, Stivala LA, Bianchi L. Anthocyanins induce cell cycle perturbations and apoptosis in different human cell lines. Carcinogenesis. 2004;25:1427–33.
- [7] Nithiya R. Food Chemistry. 5th ed. Bangkok: Odeon Store Publishing; 2002.
- [8] Department of Science Services. Anthocyanins. Information processing is ready for use. 2010;1-15.
- [9] Arusa C. Extraction and methods for analyzing anthocyanins. SWU J. Sci Technol. 2011;3:26-36.
- [10] Tena N, Asuero AG. Up-To-Date Analysis of the Extraction Methods for Anthocyanins. Antioxidants. 2022;11:286.
- [11] Bozinou E, Karageorgou I, Batra G, Dourtoglou VG, Lalas SL. PEF extraction and antioxidant activity of Moringa oleifera leaves. Beverages. 2019;5:8.

- [12] Kantala C, Supasin S, Intra P, Rattanadecho P. Evaluation of pulsed electric field and conventional thermal processing for microbial inactivation in Thai orange juice. Foods. 2022;11:1-10.
- [13] Eugene V, Nikolai IL. Extraction from foods and biomaterials enhanced by pulsed electric energy. Innov Food Sci Emerg Technol. 2016:31-56.
- [14] Eshtiagh MN, Knorr D. High electric field pulse pretreatment: potential for sugar beet processing. J. Food Eng. 2002;52:265-72.
- [15] Rastogi NK, Eshtiaghi MN, Knorr D. Accelerated mass transfer during osmotic dehydration of electrical field pulse pretreated carrots. J. Food Sci. 2006; 64: 1020-23.
- [16] Bobinaite R, Pataro G, Lamanauskas N, Satkauskas S, Viskelis P, Ferrari G. Application of pulsed electric field in extraction of blueberry juice and bioactive compounds. J. Food Sci Technol. 2015; 52: 5898-905.
- [17] Zhi-Hong Z, Lang-Hong W, Xin-An Z, Zhong H, Man-Sheng W. Effect of pulsed electric fields on pigments extracted from spinach. Innov Food Sci Emerg Technol. 2017;43:26–34.
- [18] Francisco JS, Elisa L, Juan JC, Javier R, Maria PA. Aqueous extraction of polyphenols from borage leaves enhanced by PEF. Ind Crops Prod. 2015;65:390–6.
- [19] Diego R, Maria EV, Elisa L, Javier R, Esther A. PEF as a green technology for extraction from thinned peach by-products. Innov Food Sci Emerg Technol. 2018;45:335-43.
- [20] Suthira M, Praban R. Quantity of phenolic compounds and flavonoids. KKU Sci J. 2016;44:142-52.
- [21] Tonutare T, Moor U, Szajdak L. Strawberry anthocyanins differentiation by pH differential spectroscopic method. Acta Sci Pol Hortorum Cultus. 2014;13:35-47.
- [22] Kanchana S, Samaiphon P, Apinya A, Rapikorn C, Phonpat SA, Panadda C, Kulawadee P. Analysis of antioxidant substances in rice. PSRU J. Sci Technol. 2019;4:95-108.



- [23] Rubashvill I, Tsitsagi M, Tsitsishvili V, Kordzakhia T, Ebralidze K, Buzariashvili M, Khachidze M. Sequential extraction and HPLC analysis of total anthocyanins of grape skin. The Chemist. 2019;91:33-41.
- [24] Chittapun S, Jonjaroen V, Khumrangsee K, Charoenrat T. C-phycocyanin extraction from cyanobacteria by freeze-thaw and pulsed electric field. Algal Res. 2020;46:101789.
- [25] Jansom C, Skulkhu E, Jansom V, Lerdvuthisopon N, Bhamarapravati K. Extraction of antioxidant compounds from black glutinous rice. Thammasat Med J. 2016;16(4):625-633.
- [26] Raso J, Frey W, Ferrari G, Pataro G, Knorr D, Teissie J, Miklavčič D. Recommendations on PEF reporting. Innov Food Sci Emerg Technol. 2016;37:312–21.

- [27] Mahnič-Kalamiza S, Vorobiev E, Miklavčič D. Electroporation in food processing. J. Membr Biol. 2014;247(12):1279-1304.
- [28] Zhang ZH, Wang LH, Zeng XA, Han Z, Wang MS. Effect of PEFs on spinach pigments. Innov Food Sci Emerg Technol. 2017;43:26–34.
- [29] Pataro G, Bobinaite R, Bobinas C, Satkauskas S, Raudoni R, Visockis M, Ferrari G, Viskelis P. Extraction of anthocyanins from blueberry. Food Bioprocess Technol. 2017;10(9):1595-1605.
- [30] Delso C, Berzosa A, Sanz J, Alvarez I, Raso J. Response Surface Methodology for extraction conditions. Foods. 2022;11(4):621.
- [31] Salee N, Chaiyana W, Yawootti A, Naruenartwongsakul S, Klangpetch W, Walter P, Utama-ang N. Optimization of PEF extraction of black rice. Sci Rep. 2022;12:6459.